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REMARKS

Claims 1-5 are currently pending in the application. Claim 2 has been canceled. Claims 1, 3, 4, and 5 have been amended. Support for these amendments can be found throughout the specification and claims as originally filed.

Accordingly, no new matter has been added by the current amendments. Moreover, the claim amendments requested herein should in no way be construed as acquiescence to any of the rejections and have been made solely to expedite prosecution of the application. Applicants reserve the right to pursue the claims as originally filed and/or prior to amendments made herein in this or a separate application(s).

Rejections under 35 U.S.C. §102(e)

Claims 1-5 have been rejected as being anticipated by Sayegh, *et al.* (U.S. Patent No. 6,280,957). Specifically, the Office Action states that

> Sayegh, *et al.* teach methods of transplanting grafts, including intestines with blockers of the CD28-B7 interactions, including anti-B7-1 and/or anti-B7-2 antibodies and immunosuppressive agents capable of inactivating T cells.....The claimed functional limitations would be inherent properties of the referenced methods to transplant intestines with combination therapies, including the use of anti-B7-1 and anti-B7-2 antibodies and rapamycin (Paper No. 11, page 2).

Applicants respectfully traverse this rejection. However, solely in the interest of expediting prosecution, the claims have now been amended to provide methods of down-modulating and immune response to an intestinal allograft by administering a combination of an anti-B7-1 antibody, an anti-B7-2 antibody, and a rapamycin compound. Sayegh, *et al.* teach administration of an antibody that binds B7-1 and an antibody that binds B7-2 with rapamycin, ***in combination with intravenous injection of donor hematopoietic stem cells and, optionally, an inhibitor of CD40:CD40L interaction***. Sayegh, *et al.* neither teach nor suggest administration of an antibody that binds B7-1 and an antibody that binds B7-2 to a subject in the absence of donor hematopoietic stem cells. In fact, Sayegh, *et al.* teach that administration of CTLA4-Ig

alone provides no protection from allograft rejection in their mouse model (Figure 1, discussed in Example 1, column 11). Similar results are also disclosed for skin graft rejection (Figure 2, and columns 11 and 12).

In view of the foregoing remarks and amendments, it is respectfully requested that the rejection of the claims as anticipated by Sayegh, *et al.* be reconsidered and withdrawn.

Rejections under 35 U.S.C. §103(a)

Claims 1-5 have been rejected as obvious over deBoer, *et al.* (U.S. Patent No. 5,869,050), in view of Lenschow, *et al.* (*Transplantation* (1995) 60:1171), Tarumi, *et al.* (*Transplantation* (1999) 67:520) and/or Newell, *et al.* (*J. Immunol.* (1999) 163:2358). More specifically, the Office Action states:

> Given that CTLA4IG blocks both B7-1 and B7-2-mediated responses and given the combination of anti-B7-1 and anti-B7-2 antibodies achieve significant inhibition of allogenic responses and graft rejection, one of ordinary skill in the art would have been motivated to combine both B7-1-specific and B7-2-specific antibodies to inhibit transplant rejection, including intestinal rejection. Given the teachings of deBoer, *et al.*, Lenschow, *et al.*, Tarumi, *et al.*, and Newell, *et al.*, the ordinary artisan would have an expectation of success in prolonging intestinal graft survival by blocking both B7-1 and B7-2 mediated interactions.

This rejection is respectfully traversed. To establish a *prima facie* case of obviousness for the claimed invention, there must have been some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings in the manner proposed by the Examiner. Second, there must have been a reasonable expectation of success at the time the invention was made. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Applicants submit that, at the time the invention was made, there was no motivation to combine the art in the manner suggested by the Examiner to arrive at the claimed invention. Specifically, prior to the instant disclosure, one of ordinary skill in the

art would not have been motivated to target signaling by B7-1 and B7-2 to prolong intestinal allograft transplants. As previously presented by Applicants, the nature of the immune response to intestinal allografts is unique compared to other types of allografts and, thus, results obtained from experiments with other types of allografts cannot be extrapolated to transplantation of intestinal grafts. In particular, it is widely held by experts in the community that "[i]n clinical and experimental transplantation, the intestine is the most immunogenic organ among all the solid organs" (Zhang, Z., *et al.* (1996) *Transplantation* 62:1267; page 1271, 3rd paragraph, left column; Appendix A), "rejection is an almost uniform occurrence after small bowel transplantation (Sudan, D., *et al.* (2000) *Am. J. Gastroenterol.* 95:1506; page 1512, 3rd paragraph, right column; Appendix B), and therapies which are effective at preventing transplant rejection of allografts of other types of tissues are ineffective in the prevention of small bowel allograft rejection (Yin, *et al.* (1996) page 1537, first full paragraph, right column).

In view of the state of the art at the time the present application was filed, the art cited by the Examiner fails to provide the necessary motivation which would enable the skilled artisan to make and use the claimed invention with any expectation of success. Specifically, deBoer, *et al.* teach that a combination of anti-B7-1 antibodies and cyclosporine A ***completely block T cell activation*** in a mixed lymphocyte culture assay (see, column 14, lines 1-10). Based on these data, deBoer, *et al.* generally propose treating transplant rejection using a combination of anti-B7-1 antibodies and an immunosuppressive agent. However, deBoer, *et al.* did not test any immunosuppressive agent other than cyclosporine A, nor do they teach or suggest treatment of transplant rejection using B7-2 antibodies. Moreover, deBoer, *et al.* do not specifically teach the treatment of intestinal allografts as taught by the instant specification.

Lenschow, *et al.* teach that B7-2 was first discovered based on the inability of anti-B7-1 mAbs to significantly block allogenic mixed lymphocyte reactions *in vitro.* They further teach that anti-B7-2 mAb, either alone or in association with anti-B7-1 mAb, suppresses alloreactivity *in vitro* and *in vivo*, and that the combination of anti-B7-1 and anti-B7-2 mAbs significantly prolongs the mean survival time of allografts of pancreatic islet cells. However, Lenschow, *et al.* do not teach or suggest treating any type of allograft rejection with a combination of anti-B7-1 and anti-B7-2 mAbs plus

another agent, let alone a rapamycin compound. Nor do Lenschow, *et al.* teach or suggest the treatment of intestinal allografts as taught by the instant specification.

Accordingly, the teachings of deBoer, *et al.* or Lenschow, *et al.* taken either separately or in combination do not teach that the claimed combination of an anti-B7-1 antibody, an anti-B7-2 antibody and a rapamycin compound should be used to treat or prevent any type of allograft rejection, let alone rejection of an intestinal allograft. Indeed, this rejection appears to be based on the supposition that if the combination of anti-B7-1 mAbs and cyclosporin A works *in vitro* on mixed lymphocytes (as taught by deBoer, *et al.*), and the combination of anti-B7-1 and anti-B7-2 works *in vivo* on pancreatic islet cells (as taught by Lenschow, *et al.)*, then one of ordinary skill would be motivated to combine the teachings of these references because the combination of all three molecules would be expected to work better that either combination of two molecules taught by these references. Applicants respectfully disagree.

The very teachings by deBoer, *et al.*, that the combination of anti-B7-1 mAbs and cyclosporin A ***completely block T cell activation***, would not motivate one of ordinary skill in the art to try additional inhibitors because they would not expect additional inhibitors to provide any further benefit. Thus, deBoer, *et al.* actually teach away from the claimed invention. It is well established that there can be no suggestion to combine references if a reference teaches away from its combination with another source. *E.g.*, see *Tec Air, Inc. v. Denso Manufacturing Michigan Inc.*, 192 F.3d 1353, 52 USPQ2d 1294 (Fed. Cir. 1999).

As stated by the court in *ATD Corp. v. Lydall, Inc.* 159 R.3d 534, 48 USPQ 1321 (Fed. Cir. 1998):

> There must be a teaching or suggestion within the prior art, or within the general knowledge of a person of ordinary skill in the field of the invention, to look to particular sources of information, to select particular elements, and to combine them in the way they were combined by the inventor.

Applicants respectfully submit that this motivation to make the particular combination presently claimed is simply not found within the teachings of either deBoer, *et al.* or Lenshow, *et al.*

Moreover, the disclosures of both Newell, *et al.* and Tarumi, *et al.* do not provide the motivation lacking in the teachings of deBoer, *et al.* and Lenschow, *et al.* In particular, the interpretation of Newell, *et al.* presented in the Office Action is incorrect. Indeed, contrary to the assertion that Newell, *et al.* teach that CTLA4Ig prevents intestinal allografts, the data presented by Newell, *et al.* actually demonstrate that administration of CTLA4-Ig ***does not*** block intestinal allograft rejection in wild type mice (page 2358, column 2, first paragraph, line 21-23, also page 2359, column 2, first paragraph to page 2360, column 1, first paragraph, and also figure 1). In actuality, inhibition of transplant rejection was reported by Newell, *et al.* to occur only as a result of administration of CTLA4-Ig to CD8 knock out mice (page 2358, column 2, first paragraph, line 21-23, and page 2360, column 2, second paragraph).

The ineffectiveness of CTLA4Ig in prolonging intestinal allograft transplants, as taught by Newell, is further corroborated by the teachings of Yin, *et al.* Yin, *et al.* also teach that the inhibition of the CD28/CTLA4 pathway with CTLA4-Ig was insufficient to inhibit transplant rejection of small bowel allografts in their model system. Accordingly, the teachings of these two references demonstrate that one approach to inhibit the rejection of intestinal allografts, *i.e.*, by using CTLA4-Ig, failed.

Tarumi, *et al.* is the only reference cited in the Office Action that teaches a detectable positive effect of CTLA4Ig on intestinal allograft survival. In addressing the discrepancy between their data and those of Yin, *et al.*, Tarumi, *et al.* concluded that "[w]e used a relative low-responder Lewis/Brown-Norway combination; however, Yin, *et al.* used the high-responder Lewis/ACI combination." Similar to the Newell, *et al.* experiments above, these rats are not wild-type animals. Thus, Tarumi, et al. merely confirm what Newhall, *et al.* and Yin, *et al.* already taught; that in rats that are not low responders, the CTLA4Ig treatment will not work. Moreover, Tarumi, *et al.* do not teach or suggest a solution to this problem. Nor do they even attempt to predict if combinations of molecules might be successful in inhibiting intestinal allograft rejection, let alone what particular combinations might actually work. Accordingly, the teachings of Tarumi, et al. do not cure the deficiencies of Newall, *et al.*

Prior to Applicants demonstration that B7-1 and B7-2 expression by recipient cells was required for transplant rejection using knock out animals, the solution to the

problem of inhibiting the rejection of intestinal allografts remained elusive. Applicants findings were significant in that they demonstrated for the first time that specific targeted inhibition of B7-1 and B7-2 (*e.g.*, via administration of blocking antibodies to these two molecules) in combination with a rapamycin compound was required in order to achieve a therapeutically effective reduction of the immune response to an intestinal allograft . Applicant's success in achieving what the art failed to do cannot be rendered obvious in view of that same art.

Moreover, even if it might be argued that one of ordinary skill in the art would have been motivated to modify the teachings of the cited references to try various combinations of molecules, the cited references lack sufficient guidance which would enable the skilled artisan to predict which combinations, if any, would be successful. As discussed previously, the rejection of intestinal type allografts is a process which is biologically distinct from the rejection of other tissue and organ grafts. At the time of the invention, the ordinarily skilled artisan was aware of this distinction and recognized that teachings in the prior art which were not specific to intestinal allografts. For example, the teachings of deBoer, *et al.*, and the teachings of Lenschow, *et al.,* were simply not sufficient to provide a reasonable expectation of success when applied to the prevention of intestinal allograft rejection.

One or ordinary skill in the art would also have recognized that the remaining disclosures cited by the Examiner (Tarumi, *et al.* and Newell, *et al.*,) which specifically relate to intestinal type allografts, did not compensate for the deficiencies of the disclosures of deBoer, et al. and Lenschow, *et al.* The Newell disclosure teaches that administration of CTLA4-Ig is insufficient to block intestinal allograft rejection in wild type mice, as discussed above. Tarumi, *et al.* teach that this therapy does not work in mice with normal responsiveness. Since CTLA4-Ig is known to block B7-1 and B7-2 induced costimulation, this suggests that blockage of B7-1 and B7-2 is insufficient to prevent intestinal allograft rejection.

Although the Tarumi disclosure presents evidence that CTLA4-Ig can prevent intestinal allograft rejection in one model system, this is not corroborated by the prior art, given the disclosure of Newell, *et al.* and also Yin, *et al.*, and Sayegh, *et al.*, discussed above, each of which were known to the ordinary skilled artisan art at the time of the

present invention. Thus, at the time the invention was made, there were known teachings in the art which were contradictory to the teachings of Tarumi, *et al.* and to the position of the Examiner. This controversy in the art at the time of the invention indicates that one or ordinary skill in the art would not have had a reasonable expectation of success at making the claimed invention.

Furthermore, even in the presence of teachings in the art which showed that administration of CTLA4-Ig was sufficient to inhibit intestinal allograft rejection in a low responder mouse model, the ordinarily skilled artisan would not reasonably have expected that specifically targeting B7-1 and B7-2 mediated signaling, e.g., by utilizing blocking antibodies, would produce the same results as use of the CTLA4-Ig molecule, prior to the immediate disclosure. This is true because CTLA4 binds multiple ligands and CTLA4-Ig therefore blocks a wider range of signaling molecules than B7-1 and B7-2 specific antibodies. Applicants' invention is based on a specific analysis of the involvement of B7-1 and B7-2 in intestinal allograft transplant rejection, made in a highly relevant mouse model system. Applicants' findings conclusively indicate that B7-1 and B7-2 expression by recipient cells is required for transplant rejection, since mice that were deficient in B7-1 and B7-2 exhibited significantly reduced rejection. These findings were significant in that they indicated that blockage of B7-1 and B7-2 signaling was sufficient to inhibit transplant rejection in a highly relevant model system. Prior to these findings, there was no indication that blockage of B7-1 and B7-2 signaling was sufficient, given that the CTLA4-Ig molecule was not a specific inhibitor of B7-1 and B7-2 (e.g., it was also taught to inhibit alternative costimulatory receptors which bind CTLA-4 (e.g., B7-3 (Boussiotis, *et al.*, *PNAS USA 90:* 11059-63 (1993)). Applicants' determination of the requirement for B7-1 and B7-2 in intestinal type allograft transplant rejection, coupled with exemplification of allograft survival from administration of anti-B7-1 and anti-B7-2 antibodies, ***conclusively*** demonstrated the therapeutic efficacy of the claimed methods.

In short, none of the cited references alone or in combination suggest the use of a combination of rapamycin with at least two B7 antibodies as presently claimed. At best, the cited references might be viewed as providing the suggestion, *e.g.,* piquing a scientist's curiosity, to try various combinations of immunosuppressive antibodies and

immunosuppressive agents. However, this is not the standard required to establish obviousness under 35 U.S.C. §103. See *In re Dow Chemical Cp.,* 837 F.2d 469 (Fed. Cir.1988). "'Obvious to experiment' is not a proper standard for obviousness". And see *In re Eli Lilly & Co.,* F.2d 943 (Fed. Cir. 1990) "A 'obvious to try' situation exists when a general disclosure may pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure"

In light of the above, Applicants maintain that the Examiner has not only failed to demonstrate that the motivation to combine the cited references existed at the time of the invention, by has also failed to demonstrate that their combined teachings provide a reasonable expectation of success to the ordinary skilled artisan at the time the invention was made. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

SUMMARY

In view of the remarks set forth above, it is respectfully submitted that this application is in condition for allowance. It there are any remaining issues or the Examiner believes that a telephone conversation with Applicants' attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicant's attorney at (617) 227-7400.

Respectfully submitted,

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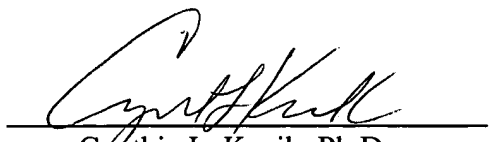

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Appendix A

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PATTERN OF LIVER, KIDNEY, HEART, AND INTESTINE ALLOGRAFT REJECTION IN DIFFERENT MOUSE STRAIN COMBINATIONS[1]

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With advances in microsurgery and molecular biology, the mouse model for organ transplantation has become increasingly popular. However, knowledge about these models is limited, as only a small number of c nters have experience with murine models. In this study, we compared the rejection pattern after liver, kidney, heart, and small bowel transplantation in the three different mouse strain combinations: (1) C57BL/6 ($H2^b$)→BALB/c ($H2^d$), (2) BALB/c ($H2^d$)→CBA ($H2^k$), and (3) C57BL/6→C3H/HeN ($H2^k$).

Our study demonstrated that mouse allograft survival varies depending on the organ graft and on the donor-recipient strain combinations. The majority of liver allografts were spontaneously accepted despite complete MHC disparity. A mixed pattern of acute rejection and acceptance occurred in kidney recipients depending on the donor-recipient strain combination. All the heart grafts developed rejection and all the intestinal grafts were rapidly rejected with no spontaneous acceptance. The criteria for rejection, the potential applications, and the limitations of each model are discussed. Th models d scribed in this article provide a number of useful choices for organ transplantati n r search.

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The murine solid organ transplant model has become increasingly popular (*1–11*). The mouse model offers several advantages for transplantation immunology research. First,

many congenic, transgeneic, and knockout strains are available; second, molecular biology probes and techniques are more widely available for the mouse than for other laboratory animals; and third, mice are relatively inexpensive. With state-of-the-art microsurgical techniques, virtually all solid organ transplants performed in man, including heart, kidney, liver, and intestine, can also be performed in mice with a high success rate (*8, 11–14*). Despite the enthusiasm of using the mouse model, the unique rejection pattern in each organ graft and the application and limitation of these models have not been well documented. The present study compared the rejection pattern after liver, kidney, heart, and small bowel transplantation in the three different mouse strain combinations. The criteria for rejection in these grafts and the application and limitation of each model were discussed.

MATERIALS AND METHODS

Animals. Male inbred mice strains including C57BL/6 ($H2^b$), BALB/c ($H2^d$), C3H/HeN ($H2^k$), CBA/J ($H2^k$), and C57BL/10 ($H2^b$) were supplied by Harlan Sprague-Dawley (Indianapolis, IN) and housed under conventional conditions at the Animal Care Facility, University of Western Ontario. Mice weighing between 25 and 30 g were used for all experiments. Animals were cared for in accordance with our institution's guidelines for experimental animals.

Experimental groups. The liver, kidney, heart, and intestine transplantations were performed in three different mouse strain combinations. Each group included 5–12 animals per organ transplant. These donor-recipient strain combinations provide a strong histocompatibility barrier due to mismatching in both major and minor MHC antigens (*15*).

Surgical models. All donors and recipients were given atropine (0.04 mg/kg) and buprenorphine (0.05 mg/kg) by subcutaneous injection before being anesthetized with an intraperitoneal injection of pentobarbital (65 mg/kg).

The orthotopic liver transplantation was carried out using the technique developed by Qian et al. (*13*). After recipient hepatectomy, revascularization was accomplished by a combination of suture and cuff techniques. The hepatic artery was not reconstructed. Cholecystectomy was performed, and bile duct patency was assured with a fine polyethylene tube stent.

Kidney transplants were performed following the procedure previously reported by our group (*8*). The left kidney attached to a segment of the aorta and the renal vein along with ureter and bladder patch were removed en bloc. The donor aorta and inferior vena cava were then anastomosed end to side to the recipient abdominal aorta and inferior vena cava below the level of the native renal vessels, respectively. The native right kidney was removed before revascularization. Donor and recipient bladders were anastomosed dome to dome. The native left kidney was removed 7 days after grafting.

The surgical technique for intra-abdominal heterotopic heart transplantation was previously described by Corry and Russell (*12*). Briefly, a median sternotomy was performed in the donor, and the right and left superior vena cava were ligated. The ascending aorta and pulmonary artery were transected, and all pulmonary veins were ligated en bloc. The ascending aorta and pulmonary artery of the donor were anastomosed end to side to the recipient aorta and inferior vena cava, respectively.

Vascularized heterotopic intestine transplants were carried out using the technique developed in our center (*10*). Briefly, the proximal segment of jejunum was isolated by ligating mesenteric vessels and removing duodenum, ileum, and entire colon. The portal vein was mobilized by dividing the pyloric and splenic veins. The aorta was exposed after the renal arteries and celiac trunk were ligated. The graft was perfused in situ with cold, heparized, lactated Ringer's solution via the infrarenal aorta. After the portal vein was divided close to the liver hilum, the graft was removed with a patch of aorta and stored in lactated Ringer's solution at 4°C. End-to-side anastomoses were performed between donor aorta and recipient aorta, and between donor portal vein and recipient inferior vena cava. Both ends of the graft were exteriorized as stomas. The native intestine was left intact.

Host/graft survival. The mice were followed up and weighed daily until the endpoint of rejection or until they were killed on postoperative day (POD*) 100. A full necropsy was performed to determine the cause of death for each animal. Death within 3 days after surgery (approximately 10%) was considered a technical failure and was excluded from the data analysis. Host survival was used as the endpoint of rejection for orthotopic liver and kidney transplants, in which the graft rejection led to host death. Graft survival was used as the endpoint of rejection for heterotopic heart and intestine transplants, since a nonfunctioning graft due to rejection may not affect survival of the recipient (*10, 12*). To further study the correlation between intestinal graft and host survival, additional animals (6–10 per group) were followed until they died or were killed on POD 28. In addition, two to three animals in each group were killed at different intervals (1 week to 1 month) for sequential histological studies.

Criteria for heart graft rejection. Direct abdominal palpation of heterotopically transplanted hearts was used for assessing the graft viability. The pulsation of heart grafts was monitored daily by two independent observers who were blind to the experimental design. The degree of pulsation was scored as A, beating strongly, B, noticeable decline in the intensity of pulsation, or C, complete cessation of cardiac impulses. Animals were killed when cardiac impulses were no longer palpable. At the premorbid laparotomy, the contraction of hearts was directly observed and the graft was removed for histological analysis.

Criteria for intestinal graft rejection. Clinical signs of intestinal rejection were recorded daily by two independent observers who were blind to the experimental design. Clinical signs of intestinal rejection included an increasing output of mucus from stomas, necrosis or closure of stomas, and development of palpable mass. The signs of rejection were scored as A, no change, B, mild change, or C, marked change. Two or more signs of marked changes were considered to be end-stage rejection. The animals were then killed, and necropsy and histological studies were performed.

Pathology. At necropsy, tissue samples were harvested, fixed in 10% buffered formaldehyde, embedded in paraffin, and then stained with hematoxylin-eosin. The microscopic sections were examined for evaluation of rejection by a pathologist (B.G.) blind to the study. The severity of rejection was scored as: 0, no change; 1+, mild-to-moderate change; or 2+, marked change (*16*).

Graft functions. Before mice were killed, serum was collected for liver function tests (albumin, total protein, bilirubin, aspartate aminotransferase, and alanine aminotransferase) and a renal function test (creatinine). Electrocardiograph (EKG) monitoring was conducted in selected animals with heart grafts.

Statistical analysis. The data are reported as the mean±SEM. Survival data were compared using the rank-log test. Histological findings were compared using the Mann-Whitney *U* test. Differences with *P*-values less than 0.05 were considered to be significant.

RESULTS

Liver transplants. Table 1 shows the survival data of liver allografts in different strain combinations. None of the 25 recipients developed acute rejection during the first 4 weeks. Most of the liver allografts in all the strain combinations were spontaneously accepted despite a complete MHC mismatch. These animals were well until they were killed on

* Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; EKG, electrocardiogram; POD, postoperative day.

TABLE 1. Survival of liver allografts in different mouse strain combination

Strain combinations	n	Survival (days)	Acceptance (%)
C57BL/6→BALB/c	7	30, 32, >100 × 5	72
BALB/c→CBA	7	50 × 2, 63, >100 × 4	57
C57BL/6→C3H/HeN	11	30, 32, 50, >100 × 8	73

TABLE 3. Survival of kidney allografts in different mouse strain combinations

Strain combinations	n	Survival (days)	Acceptance (%)
C57BL/6→BALB/c	10	8 × 3, 9, 10, 28, 59, 63, >100 × 2	20
BALB/c→CBA	6	8, 9 × 2, >100 × 2	33
C57BL/6→C3H/HeN	6	8, 9, 9, >100 × 3	50

POD 100. The rate of spontaneous acceptance varied depending on the donor-recipient strain combinations: 72% for B6→BALB/c, 57% for BALB/c→CBA, and 73% for B6→C3H/HeN. The remaining animals developed fatal liver rejection from POD 30 to 77. These animals had weight loss, enlarged liver, jaundice, and markedly elevated transaminases (data not shown). Table 2 shows the histological changes in the liver grafts. Randomly killed animals on POD 30 demonstrated mild to moderate liver rejection, including lymphocytic infiltration, endothelialitis, arteriolitis, bile duct infiltration, and hepatocellular degeneration. Graft histology in animals killed on POD 100 showed more subtle features of rejection. Interestingly, evidence of chronic rejection, including fibrosis and bile duct proliferation, was seen in these animals. Moderate elevation of ALT, AST, and bilirubin levels was found in long-term survivors on POD 100 (AST, 391±86 μm/L; ALT, 216±24 μm/L; bilirubin, 39±11 μmol/L).

Kidney transplants. Table 3 shows the survival of kidney allografts in different combinations. Twenty percent of group 1, 33% of group 2, and 50% of group 3 grafts were spontaneously accepted with slightly elevated creatinine levels (creatinine, 70±4 μmol/L) on POD 100. The remaining grafts were rapidly rejected between 8 and 10 days with marked elevation of creatinine levels (creatinine, 120±20 μmol/L). The rate of acceptance varied depending on the donor-recipient strain combination.

Table 4 shows the histological changes of kidney allografts in the different strain combinations. Mice that died on PODs 8–10 showed features of acute cellular and vascular rejection, including lymphocytic infiltration, hemorrhage and edema in interstitium, tubulitis, vasculitis, and glomerular and tubular necrosis. Animals surviving to POD 100 showed typical features of chronic rejection, including interstitial fibrosis, tubular atrophy, and intimal proliferation.

Heart transplants. Table 5 shows survival of heart allografts in different mouse strain combinations. All the heart grafts developed acute rejection, with a mean survival of 9.6±0.4 days for group 1, 13.6±1.3 days for group 2, and 14.3±0.9 days for group 3. The tempo of rejection varied depending on the strain combination. Histologic examination showed that the rejected hearts developed severe vascular and cellular rejection, as demonstrated by the presence of lymphocytic infiltration, vasculitis, infarction, ischemia, and thrombosis (Table 6).

Intestinal transplants. Table 7 shows intestinal graft survival in different mouse strain combinations. All intestinal grafts were rapidly rejected, with a mean graft survival of 8.3±1.6 days for group 1, 8.6±0.9 days for group 2, and 8.1±0.8 days for group 3. There was no statistical difference among different strain combinations ($P>0.05$). Table 8 shows the host survival of intestinal allografts in different strain combinations. There was no correlation between graft survival and host survival in any group ($P>0.05$). Forty percent to 50% of mice in each group died from fatal rejection between PODs 6–13. The typical clinical findings for these animals were characterized by hunched postures, dehydration, emaciation, and necrotic stomas. In the remaining animals, the general condition was not affected despite clinical signs of rejection with a palpable abdominal mass. Necropsy in these animals killed on POD 28 showed that the graft had developed into a firm, encapsulated, nodular mass, and the intestinal graft lumen was filled with caseous fluid with no villi appearing.

Table 9 shows the histological changes in the intestinal grafts at necropsy. Mice that died from fatal rejection on POD 7–10 had the typical features of acute rejection, including frequent mitoses, loss of goblet cells, cryptitis, and lymphocytic infiltration with flattening and sloughing of villi. The mice killed on POD 28 also showed evidence of rejection, with more advanced features than those seen in the former group. The rejection was characterized by complete mucosal destruction and full-thickness necrosis.

DISCUSSION

The mouse heart and kidney transplant models that were developed 20 years ago and the liver and small bowel transplant models, developed in the 1990s, have provided an excellent opportunity to study transplantation immunology (*1–14*). However, knowledge about these models was limited because only a small number of centers have experience with murine models. This study compares the rejection pattern of

TABLE 2. Median scores of histological changes in liver allografts on POD 30 and POD 100

	POD 30			POD 100		
	B6→BALB/c	BALB/c→CBA	B6→C3H	B6→BALB/c	BALB/c→CBA	B6→C3H
Lymphocytic infiltration	2.0	1.5	1.0	1.0	1.5	1.5
Endothelialitis	1.5	1.5	1.0	0.5	1.0	1.0
Arteriolitis	0.5	0.5	0	0	0	0
Bile duct changes[a]	2.0	1.0	0.5	1.5	1.0	1.5
Hepatocytic changes[b]	2.0	1.0	0.5	0	1.0	0

[a] Bile duct changes refer to proliferation and lymphocytic infiltration.
[b] Hepatocytic changes refer to degeneration and necrosis.

TABLE 4. Median scores of histological changes in kidney allografts

	PODs 8–10			POD 100		
	B6→BALB/c	BALB/c→CBA	B6→C3H	B6→BALB/c	BALB/c→CBA	B6→C3H
Interstitial changes						
Lymphocytic infiltration	1.0	2.0	2.0	2.0	1.5	1.5
Hemorrhage	1.0	2.0	1.0	0	0	0
Edema	1.5	2.0	1.5	1.0	0	1.5
Fibrosis	0	0	0	1.0	1.5	1.0
Tubular changes						
Tubulitis	1.0	1.0	1.5	1.5	1.5	2.0
Tubule necrosis	2.0	2.0	1.5	1.5	0.5	1.5
Glomerular changes						
Necrosis	1.0	2.0	0.5	1.0	0.5	0.5
Vasculitis	1.0	1.0	2.0	1.5	1.5	1.0

TABLE 5. Survival of heart allografts in different mouse strain combinations

Strain combinations	n	Mean survival (days)	Individual survival (days)
C57BL/6→BALB/c	14	9.6±0.4	8 × 3, 9 × 6, 10, 11 × 3, 14
BALB/c→CBA	6	13.6±1.3	10, 11, 13, 14, 15, 19
C57BL/6→C3H/HeN	11	14.3±0.9	10, 11, 13 × 3, 14 × 2, 15 × 2, 19, 20

TABLE 6. Median scores of histological changes of heart allografts at necropsy

	B6→BALB/c	BALB/c→CBA	B10→C3H
Lymphocytic infiltration	1.5	1.0	1.0
Vasculitis	2.0	1.5	2.0
Infarction	2.0	1.5	2.0
Ischemia	1.5	2.0	2.0
Thrombosis	2.0	2.0	2.0

TABLE 7. Intestinal graft survival in different mouse strain combinations

Strain combinations	n	Mean survival (days)	Individual survival (days)
C57BL/6→BALB/c	8	8.3±1.6	6 × 3, 7 × 3, 8, 19
BALB/c→CBA	5	8.6±0.9	6, 7, 9, 10, 11
C57BL/6→C3H/HeN	6	8.1±0.8	6, 7 × 2, 8, 10, 11

TABLE 8. Host survival of intestinal allografts in different mouse strain combinations

Strain combinations	n	Host survival (days)
C57BL/6→BALB/c	8	6, 7 × 2, 8, >28 × 4
BALB/c→CBA	7	6, 11, 13, >28 × 4
C57BL/6→C3H/HeN	10	6 × 2, 7, 11, >28 × 6

liver, kidney, heart, and small bowel transplants in the three different mouse strain combinations. We have demonstrated that mouse allograft survival varies depending on different organs and donor-recipient strain combination. The majority of the liver allografts in all the strain combinations was spontaneously acc pted despite complete MHC disparity. A mixed pattern of acute rejection and acceptance occurred in kidney recipients depending on the donor-recipient strain combination. All the heart grafts developed acute rejection, and all the intestinal grafts were rapidly rejected with no spontaneous acceptance. It has become increasingly clear that the intensity of rejection is organ dependent (*17*, *18*). These data further support this concept in vascularized murine transplants.

Mice with liver allografts did not develop acute rejection. Most of liver recipients survived indefinitely despite moderately impaired liver functions and histopathological evidence of chronic rejection. The liver has been known as a unique organ with regard to tolerance. Spontaneous acceptance of pig liver allografts was first reported by Calne et al. (*19*). Subsequently, Kamada et al. reported such acceptance occurred in the rat low-responder strain, such as DA→PVG, but not in the high-responder strain, such as ACI→Lewis (*20*). More recently, the full spectrum of "hepatic tolerogenicity" in 13 mouse strains was reported by Qian et al. (*5*). Both rat and mouse liver transplant models have been used extensively to study the mechanisms of hepatic tolerance and tolerance in general (*20*, *21*). Recently, they have shown that hepatic tolerance may be attributed to the mixture of chimerism of multilineage donor cells in the recipient tissues (*5*). More recently, Lu et al. (*22*) showed that immature dendritic cells from liver may play an important role in hepatic tolerance. The low incidence of rejection in mouse liver allografts makes this model less than ideal to study the mechanisms of rejection.

The mouse kidney transplant model was first developed by Skoskiewicz et al. (*14*), and was recently refined by our group (*8*). Russell and his colleagues (*23*) reported that spontaneous acceptance occurred in some mouse strain combinations. In this study, we found that a mixed picture of acute rejection and acceptance occurred in the strain combinations used in this study. The rate of acceptance varied depending on the donor-recipient strain combination. The orthotopic kidney transplant model provides a functional graft to study immunosuppressive agents and mechanisms of rejection. However, unlike the rat kidney transplant model, we have been unable to identify a mouse strain combination that produces uniform rejection (*2*, *7*, *23*, *24*). This limitation may be overcome by using a large sample size. Because of the variation in survival in the mouse kidney transplant model, the renal function may be an alternative endpoint for assessment of graft rejection. The long-term surviving renal allografts may provide a useful tool to study chronic rejection.

TABLE 9. Median scores of histological changes of intestinal allografts at necroposy

	PODs 7–10			POD 28		
	B6→BALB/c	BALB/c→CBA	B6→C3H	B6→BALB/c	BALB/c→CBA	B6→C3H
Mitosis	1.0	2.0	1.5	0	2.0	0
Cryptitis	1.5	1.0	1.0	2.0	0.5	0
Lymphocytic infiltration	0.5	1.0	1.0	2.0	1.0	1.0
Sloughing of villi	1.0	1.5	1.0	2.0	1.0	2.0
Flattening of villi	0.5	1.5	1.0	2.0	1.0	2.0
Goblet loss	2.0	1.5	2.0	2.0	2.0	2.0

A more uniform pattern of rejection and relatively simple surgical technique in the heart transplants made this mouse model ideal to screen new immunosuppressive drugs and to study the mechanisms of rejection (*25–27*). Several methods, including direct abdominal palpation, visual observation during laparotomy, EKG monitoring, and pathology, have been used for assessing cardiac graft rejection (*27–29*). We believe that direct abdominal palpation of the graft is the most simple and reliable form of assessing rejection. In our 300 consecutive heart allografts, laparotomy confirmed that 90% of hearts did not have visual myocardial contraction when complete cessation of cardiac impulse was observed by two independent observers using the direct palpation technique (R. Zhong, unpublished data). The remaining 10% with myocardial contraction showed a weak electrical impulse in EKG and histological evidence of severe rejection, including massive cell infiltration and tissue necrosis/infarction. Clearly, such a "beating" heart was functionally meaningless, in terms of potential clinical relevance.

The reason why the kidney recipients survive longer than the heart transplants is not clear. One explanation of this phenomena may be related to the heterotopic model of heart grafting. The heterotopic heart transplant model alters coronary flow, with only partial chambers of the heart being perfused (*30*). This results in ischemic injury to the graft, which may accelerate rejection (*31*). The observation that orthotopic heart xenografts in primates survived longer than heterotopic grafts in the same species combination supports this hypothesis (*32*). Moreover, it has been reported that even some long-term beating heterotopic hearts under immunosuppression have advanced histopathological rejection, suggesting a caution must be needed when interpreting the studies using this nonfunctional, heterotopic heart transplant model (*33*).

All the intestinal grafts with different strain combinations were rapidly rejected with no single case of spontaneous acceptance. In clinical and experimental transplantation experiences, the intestine is the most immunogeneic organ among all the solid organs (*16, 17*). Similar observations have been found in the mouse model, since some immunosuppressive agents that proved to be effective in preventing rejection of other solid organs, such as kidney and heart, did not prolong intestinal graft survival (*7*). Therefore, the mouse intestinal transplant model is a good model to study immunosuppressive agents and mechanisms of rejection.

In similarity with the rat heterotopic intestinal transplant model, advanced rejection in nonfunctional grafts did not affect the host survival in 50–60% of mice. The pros and cons of using heterotopic and orthotopic intestinal transplant models have previously been discussed extensively in the rat model (*16*). The heterotopic model appears to be the better choice for immunological research due to the relative ease of the surgery and the resulting low mortality. The orthotopic model provides a more physiological model, and graft failure leads to the animal's death, providing a well-defined endpoint of rejection. Development of the mouse orthotopic intestinal transplant model is currently underway in our laboratory. Based on our data, we recommended using graft survival instead of host survival as the endpoint of rejection in heterotopic intestinal transplantation.

In spite of recent enthusiasm for the mouse transplant model, there are some limitations for this model. First, a higher level of microsurgical skills is required to perform transplants in the mouse, particularly for small bowel, liver, and kidney models (*5, 10, 12*). Second, the intensity of graft rejection in the mouse appears less severe than that of the rat model. For example, spontaneous acceptance of liver and kidney occurs only in the rat low-responder strains, whereas it occurs frequently in any of the mouse strains (*5, 23*). Third, there is a greater variation of graft survival in mice when compared with the rat model. For example, the standard error of the mean for rat-to-mouse skin grafts under immunosuppression was 18.4 days, while it was only 2.3 days in the mouse-to-rat model (*34*).

The mouse transplant model will continue to be important in the study of immunological responses after organ transplantation. The emergence of numerous transgenic and knockout mice has provided new avenues to dissect the mechanisms of rejection/tolerance at a molecular level. The supply of monoclonal antibodies, reagents, and probes is more widely available in the mouse than any other laboratory animal. Historically, most novel therapies in transplantation were first tested in mice (*35–38*). The mouse organ transplant models are useful tools in fundamental organ transplantation research and development of novel immunosuppressive strategies. This is especially important in our current era of cost-conscious and cost-effective research. The models described in this article provide a number of choices for organ transplantation research. Knowledge gained from this article will be useful for designing research protocols and interpreting data of organ transplant research using mouse models.

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Appendix B

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Isolated Intestinal Transplantation for Intestinal Failure

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OBJECTIVE: Parenteral nutrition sustains life in patients with intestinal failure. However, some experience life-threatening complications from parenteral nutrition, and in these individuals intestinal transplantation may be lifesaving.

METHODS: This is a retrospective review of 28 consecutive isolated small bowel transplants performed in eight adults and 20 children between December 1993 and June 1998 at the University of Nebraska Medical Center.

RESULTS: The 1-yr patient and graft survivals were 93% and 71%, respectively. The causes of graft loss were hyperacute rejection (n = 1), acute rejection (n = 5), vascular thrombosis (n = 1), and patient death (n = 1). The median length of time required until full enteral nutrition was 27 days. All 28 patients have experienced acute rejection of their small bowel grafts and rejection led to graft failure in five. Jaundice and/or hepatic fibrosis was present preoperatively in 17 of the 28 recipients and hyperbilirubinemia was completely reversed in all patients with functional grafts within 4 months of transplantation. Three patients developed posttransplant lymphoproliferative disease (11%). Three recipients developed cytomegalovirus enteritis and all were successfully treated.

CONCLUSIONS: Patient survival after intestinal transplantation is comparable to parenteral nutrition for patients with intestinal failure. Better immunosuppressive regimens are needed to decrease the risk of graft loss from acute rejection. The incidence of posttransplant lymphoproliferative disorder is higher after intestinal transplantation than after other solid organ transplants and the risk of cytomegalovirus enteritis is low with the use of cytomegalovirus seronegative donors. Liver dysfunction in the absence of established cirrhosis can be reversed. (Am J Gastroenterol 2000;95: 1506–1515. © 2000 by Am. Coll. of Gastroenterology)

INTRODUCTION

Parenteral nutrition (PN) can supply the nutritional needs of patients with intestinal failure. Although most patients tolerate PN well, PN is associated with a number of life-threatening complications, including catheter-related sepsis, venous thrombosis (leading to lack of venous access), and liver dysfunction. Mortality for PN-dependent patients with intestinal failure caused by benign diseases is reported to be 5–25% per year (1, 2). Furthermore, 40–60% of infants dependent on PN because of intestinal failure develop liver disease (3). Because of the mortality and morbidity experienced by some PN-dependent patients, there has been increasing interest in isolated small bowel transplantation. We reported our preliminary results with isolated small bowel transplantation in three children in 1996 (4). All three of these children are alive 4.5–5 yr after transplantation and continue to receive all their nutrition enterally without the need for supplemental PN or intravenous fluids. Here we report our experience with 28 isolated intestinal transplant recipients.

MATERIALS AND METHODS

Forty-seven patients were evaluated and placed on the waiting list for isolated small intestinal transplantation between December 1993 and July 1998 at the University of Nebraska. During this time, 28 primary isolated intestinal transplants were performed in eight adults and 20 children. There were three patients who remained on the isolated small bowel waiting list and died 106, 121, and 257 days after initial listing, respectively. Thirteen patients whose liver function deteriorated while awaiting isolated small bowel transplantation were changed on the waiting list to combined liver/small bowel during this same time period. Of the 13 patients whose liver disease progressed, seven patients died awaiting liver/small bowel transplantation, five patients were transplanted successfully with a combined liver/small bowel graft, and one patient is currently awaiting combined liver/small bowel transplantation. The mean waiting time was substantially longer for the 13 patients whose status was changed, compared with those who underwent isolated intestinal transplantation (412 days *vs* 192 days, respectively; $p = 0.005$). Five of the seven who died after changing their status to combined liver/small bowel were small children (mean weight = 8.1 ± 3.6 kg) and six of the seven were blood type O. Demographic characteristics including gender, primary diagnosis, age, and time on the waiting list of the 28 isolated intestinal transplant recipients are summarized in Table 1.

Table 1. Demographic Characteristics and Waiting Times of the 28 Isolated Intestinal Transplant Recipients

	Adults (n = 8)	Children (n = 20)	Total (n = 28)
Age, yr			
Median	24.7	3.7	
Range	17–59	0.4–8	
Gender			
Male	4	10	14
Female	4	10	14
Primary diagnosis			
Midgut volvulus	3	7	10
Intestinal pseudoobstruction		4	4
Gastroschisis/intestinal atresia		5	5
Resection	5	1	6
Protein-losing enteropathy		1	1
Microvillous inclusion disease		2	2
Waiting time, days			
Median	117	107	107
Range	(28–608)	(7–1224)	(7–1224)

Patient Selection

Recipient evaluation for intestinal transplantation was focused on estimation of length and function of residual native intestine, the degree of liver dysfunction and portal hypertension, and identification of life-threatening complications of PN. Patients were considered candidates if the length and function of the remaining gut revealed no opportunity to wean from PN with alternative feeding strategies and if life-threatening complications of PN were identified. The degree of liver dysfunction then determined whether an isolated intestine or combined liver/small intestinal transplant was appropriate. Liver function was evaluated biochemically and clinically. Hyperbilirubinemia alone was not a contraindication for isolated intestinal transplantation in our series in the absence of cirrhosis on liver biopsy or clinical evidence of portal hypertension. Clear-cut evidence of cirrhosis on biopsy or portal hypertension (such as portal hypertensive gastropathy and gastrointestinal [GI] bleeding, progressive splenomegaly with thrombocytopenia, dilated superficial abdominal veins), however, was an indication for combined liver/small bowel transplantation.

Donor Factors

All donors were cytomegalovirus (CMV) seronegative and matched with recipients for blood type and body size. Three patients received blood group compatible but nonidentical grafts. Donor to recipient weight ratio was 0.66 ± 0.23. The donor procurement technique has been previously described and all grafts consisted exclusively of small intestine (5). Twenty-seven of the 28 donors were treated with antithymocyte globulin (ATGAM; Upjohn, Kalamazoo, MI) (30 mg/kg) and OKT3 (muromonab CD3, Ortho, Raritan, NJ) (2.5–5.0 mg) intravenously before infusion of cold University of Wisconsin solution. No intraluminal bowel preparation was performed.

Table 2. Cold Ischemia Time, Operative Time, and Transfusion Requirements for Recipients of Isolated Intestinal Transplants

	Adults Median (Range)	Children Median (Range)	Overall Median (Range)
Cold ischemia time (hr)	10.5 (8.5–13.3)	8.3 (70.0–12.3)	8.7 (7.0–13.3)
Operative time (hr)	6.7 (4.3–8.3)	4.7 (4.2–9.4)	5.4 (4.2–9.4)
Transfusion requirements, units PRBC	3.0 (0–7)	1.4* (0–5)	2.1 (0–7)

*10 cc/kg considered equivalent to 1 unit for pediatric recipients.
PRBC = packed red blood cells.

Recipient Operation

The donor superior mesenteric artery (SMA) was anastomosed to the side of the recipient infrarenal aorta. A donor iliac artery interposition graft was required in four cases where the donor SMA was short in length because of the origin of a replaced right hepatic artery. The donor portal vein was then anastomosed with the recipient portal vein confluence (n = 9), superior mesenteric vein (n = 9), inferior vena cava (IVC) (n = 9), or right renal vein (n = 1). Patients with jaundice or hepatic fibrosis underwent drainage of their allografts into the IVC or right renal vein (due to an anomalous left-sided inferior vena cava). Continuity of the bowel was established proximally by anastomosis of proximal donor jejunum to the remaining recipient proximal bowel (stomach, n = 1; duodenum, n = 6; jejunum, n = 21). The distal ileum was anastomosed to the remaining native colon in 27 of the recipients. The other recipient had formation of an end ileostomy. A stoma was brought out for biopsy access in 24/28 (proximal jejunum, n = 7; end-loop ileostomy, n = 17). The ostomy was usually taken down 1 yr (mean = 365 ± 269 days) after transplantation. Cold ischemia times, operative times, and transfusion requirements are summarized in Table 2. Cross-matches and human leukocyte antigen (HLA) typing were performed retrospectively after transplantation and no decision for use of an allograft was made based on these results (see Tables 3 and 4).

Postoperative Management

Oral tacrolimus (Prograf, Fujisawa, Deerfield, IL) and prednisone were used as primary immunosuppression. Tacrolimus levels were maintained between 20 and 30 ng/dl for the first 3 months after transplantation. Two patients were switched to cyclosporine (Neoral, Novartis, East Hanover, NJ) because of presumed tacrolimus-related cardiomyopathy (n = 1) and suspected tacrolimus-related hemolysis (n = 1). Alprostodil (Prostin VR, Pharmacia & Upjohn Company, Kalamazoo, MI) was administered intravenously for the first 7 days after transplantation. Broad-spectrum intravenous antibacterial prophylaxis was given for 1 wk and antifungal prophylaxis for 6–8 wk after transplantation. Intravenous ganciclovir (Cytovene, Roche Laboratories Inc., Nutley, NJ) was given for 2–3 wk followed by oral

Table 3. Characteristics of Individual Pediatric Recipients of Isolated Intestinal Transplants

Patient Number	Age/ Gender, (Yr/M, F)	Total Bilirubin mg/dl (Pretx)	Total Bilirubin mg/dl (4 months)	Liver Biopsy	Patient Survival, Yr	Cause of Graft Failure (PTD)	Cause of Death	Blood Type (D/R)	Cross-Match	HLA Match (A, B, DR)
1	1/M	0.3	0.8	PF	5.0			O/O	C	0, 0, 0
2	5/F	4	0.3	PF, MS	4.7			O/O	C	0, 1, 0
3	5/F	0.5	0.6		4.6			A/AB	C	1, 0, 1
4	2/F	1.1	0.8	BF	1.2	Death (442)	Sepsis/MSOF	O/O	C	
5	6/F	0.3	0.3		3.6			O/O	C	1, 0, 0
6	2/M	1.6			0.5*	HR (1)		A/A	C	
7	3/M	0.3	0.6		2.2*	CR (630)		B/B	C	0, 0, 1
8	6/F	19.8	0.4	PF, BF, CH, BP	3.0†	AR (353)		O/A	I	0, 1, 2
9	2/M	0.3	0.4		1.0	Death (368)	sepsis/hemolysis	A/A	C	1, 0, 0
10	2/F	0.7	0.6		1.9†	AR (181)		O/O	C	0, 0, 1
11	2/M	0.5	0.8	PF, BP, CH	1.8			O/O	C	0, 0, 0
12	4/F	1.3	0.5	PF	1.6			A/A	C	0, 0, 1
13	0.4/F	5.6		BF, CH	0.5*	VT (6)		B/B	C	
14	5/M	1.2	0.5	PF, BF	1.5			O/O	C	1, 1, 1
15	8/F	1.6	0.8		1.5			A/A	C	1, 0, 1
16	0.4/M	2.8	0.4	PF, BF	1.0			A/A	C	
17	7/M	1.7	0.5		1.0			O/O	C	
18	1/M	0.4	0.2	PF, CH	0.8			O/O	I	
19	2/F	0.4	0.1		0.6			O/O	C	1, 0, 0
20	4/M	7.3	0.9	PF, BF, CH	0.4			B/B	C	

* Patient survival censored 6 months after graft removed, † patient retransplanted and has functional second graft.

PF = portal fibrosis; BF = bridging fibrosis; MS = macrovesicular steatosis; CH = cholestasis; BP = bile duct proliferation; HR = hyperacute rejection; AR = acute rejection; CR = chronic rejection; VT = vascular thrombosis; C = compatible; I = incompatible; PTD = posttransplant day.

Boldface type patient number indicates recipient had liver dysfunction at the time of transplantation.

acyclovir (Zovirax, Glaxo Wellcome Inc., Research Triangle Park, NC) and intravenous CMV immune globulin (Cytogam, Medimmune, Inc., Gaithersburg, MD) for the remainder of the first year after transplantation (6).

Recipients were started on an elemental diet 5–7 days after transplantation *via* continuous infusion through a gastric or enteral tube. Oral intake was then liberalized as tolerated beginning 2 to 3 wk after surgery. PN was discontinued when patients were able to consume all of their required calories through oral intake with or without supplemental tube feedings without excessive stool output.

Protocol endoscopy with biopsies was performed twice weekly for the first 4–6 wk and in the absence of rejection were performed with decreasing frequency over the first year and when indicated by increased stool output thereafter. A specimen was processed for viral culture at each endoscopy session. Rejection was diagnosed based on histological criteria. Mild rejection was identified by preservation of villous height, increased inflammatory infiltrate of the lamina propria, and focal cryptitis (represented by intraepithelial lymphocytic infiltration and apoptosis of crypt cells; see Figs. 3*A, B*). Moderate rejection was identified by abnormal villous architecture or villous blunting (see Fig. 4), increased inflammatory infiltrate of the lamina propria, and focal cryptitis. Severe rejection was identified by ulceration of the epithelium and/or severe blunting of the few remaining intact villi and severe crypt cell destruction with debris (see Figs. 5*A, B*). Regardless of histological grade, all

Table 4. Characteristics of Individual Adult Recipients of Isolated Intestinal Transplants

Patient Number	Age/ Gender, (Yr/M, F)	Total Bilirubin mg/dl (Pretx)	Total Bilirubin mg/dL (4 months)	Liver Biopsy	Patient Survival, Yr	Cause of Graft Failure (PTD)	Cause of Death	Blood Type (D/R)	Cross-Match	HLA Match (A, B, DR)
21	28/M	4	0.6	MS, CH	3.3			O/O	C	0, 1, 0
22	24/M	1.4	0.4	CH, MS, BP	0.64	AR (150)	Line sepsis	O/B	C	0, 0, 0
23	59/F	0.6			0.6*	AR (30)		B/B	I	0, 0, 0
24	23/M	12.9	0.4	PF, BF	2.8			O/O	C	0, 1, 0
25	39/M	13.3	0.5	PF, CH	1.9			O/O	C	
26	17/F	14.1		PF, BF, CN	0.6*	AR (25)		O/O	C	2, 0, 1
27	18/F	25.8		PF, CH	8 days	Death (8)	MSOF	A/A	C	0, 0, 1
28	39/F	3.8	0.4		0.6			A/A	C	1, 0, 0

* Patient survival censored 6 months after graft removed.

PF = portal fibrosis; BF = bridging fibrosis; MS = macrovesicular steatosis; CH = cholestasis; CN = collapse and necrosis; AR = acute rejection; C = compatible; I = incompatible.

Bold-face type patient number indicates recipient had liver dysfunction at the time of transplantation.

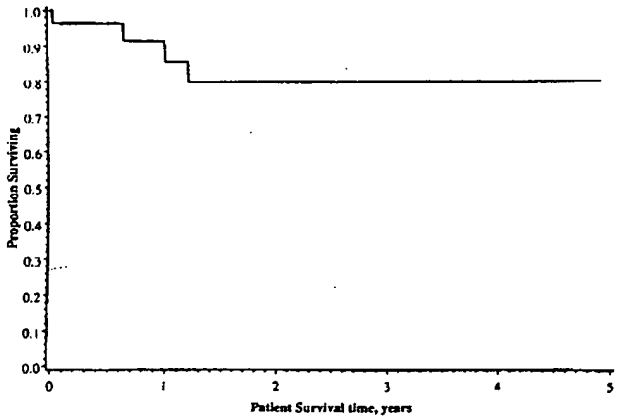

Figure 1. Kaplan-Meier graft survival in isolated intestinal transplant recipients.

rejection episodes were treated initially with bolus steroids (usually 10 mg/kg methylprednisolone intravenously daily for three doses) and an increase in the tacrolimus level by 25%. Resistant rejection episodes were treated with OKT3 (muromonab CD3) (usually 2.5 mg daily for 7–14 days in pediatric recipients).

Statistical analysis included calculation of Kaplan-Meier patient and graft survival. Univariate analysis was performed using the χ^2 test or Student's t test where applicable. Mean values are reported ± SD.

RESULTS

Patient and Graft Survival

One-year actual patient and graft survival rates were 93% and 71%, respectively. Figures 1 and 2 show the Kaplan-Meier graft and patient survival curves, respectively, for this series of patients (median follow-up = 541 days). There were eight intestinal grafts that failed in the first year after transplantation. The causes of graft loss were hyperacute rejection (n = 1), acute rejection (n = 5), patient death (n = 1), and vascular thrombosis (n = 1). Three additional bowel grafts were lost beyond the first year. Two of these three patients with late graft loss died with functioning grafts as described below, and patient 7 underwent allograft removal for chronic rejection 630 days after transplantation.

There were two patient deaths in the first year after transplantation. Patient 27 was jaundiced at the time of transplantation. Despite the absence of hepatic fibrosis, she developed coagulopathy, intraabdominal hemorrhage, and hypotension leading to multisystem organ failure. A second patient (patient 22) suffered severe anoxic brain injury after transplantation. He subsequently developed severe acute rejection and underwent explantation, but died 3 months later (234 days after transplantation) from catheter-related sepsis.

After the first posttransplant year there were two additional deaths in patients with functioning grafts. Patient 9 died secondary to complications of autoimmune hemolytic anemia unresponsive to high-dose steroids, splenectomy, plasmapheresis, or conversion to Neoral. Patient 4 died from sepsis at a hospital remote from the transplant center.

Technical complications were an uncommon cause of graft failure. Nonfunction of an allograft was not observed in this series of small bowel transplant recipients. One patient developed vascular thrombosis in this series. This event occurred in the youngest recipient (a 5-month-old jaundiced female) who received the youngest graft (10-day-old donor) and was the only jaundiced patient whose venous outflow was drained into the portal system.

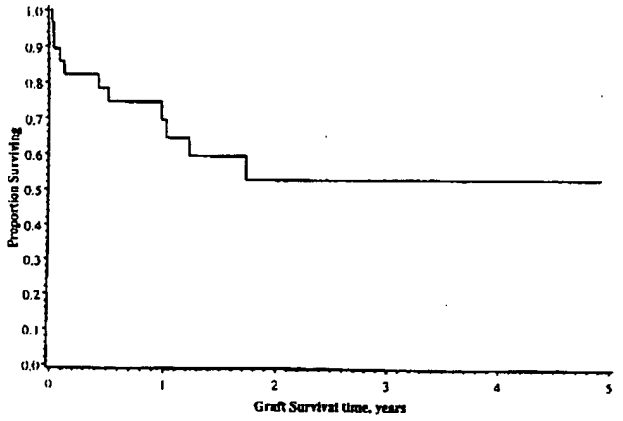

Figure 2. Kaplan-Meier patient survival for isolated intestinal transplant recipients. Patients with graft failure were censored 6 months after explantation and not routinely followed-up thereafter.

Function

All recipients (except for patients 6, 13, and 27 who had their grafts removed or died) began enteral nutrition in the first week after transplantation. The time to complete enteral nutrition was variable (range = 9–128 days, median = 27 days). Discontinuation of PN was most often delayed by the occurrence of intraabdominal abscess or viral enteritis (median = 75 days and 21 days, with and without abscess, and median = 55 days and 22 days with and without viral enteritis, respectively). Rejection episodes alone did not affect the length of time to discontinuation of PN. Twenty-three of the 28 recipients had functional bowel grafts at the time of hospital discharge, defined as complete discontinuation of parenteral nutrition. Patient 22 had discontinued PN on posttransplant day 66, but required return to PN after graft removal for uncontrolled acute rejection on posttransplant day 150.

In the four patients with intestinal pseudoobstruction, bowel continuity was established at the level of the native proximal jejunum. All have shown sufficient improved gastric motility to tolerate an oral diet with or without supplemental gastrostomy tube feedings. Temporary episodes of delayed gastric emptying have occurred on several occasions in these patients in association with acute infections or surgical procedures.

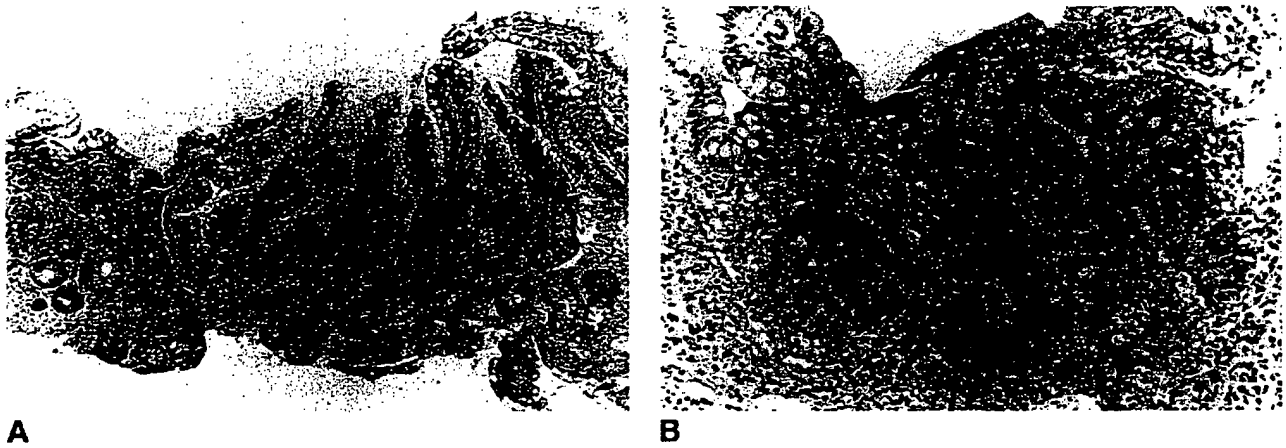

Figure 3. Mild rejection of a small intestinal graft. *(A)* On low power, the histology reveals normal villous height, but an increase in the inflammatory infiltrate of the lamina propria (magnification ×40). *(B)* The typical feature of rejection is cryptitis, identified by an intraepithelial lymphocytic infiltration of the crypts (small arrows) and apoptosis of crypt cells (large arrow) (magnification ×200).

Rejection

Sixty-one rejection episodes were identified in the first year after transplantation in 22 of 23 recipients discharged from the hospital with functional grafts. The one patient rejection-free at 1 yr after transplantation, however, subsequently developed acute intestinal allograft rejection. The median number of acute allograft rejection episodes per patient was two (range = 0–11). An average of 27 ± 10 endoscopies with biopsies were performed per patient in the first year after transplantation. The pathological severity of these rejection episodes was interpreted as minimal in five, mild in 45 (Fig. 3), moderate in seven (Fig. 4), and severe in four patients (Fig. 5). Most rejection episodes (60%) occurred in the first 3 months after transplantation. Five recipients (19%) developed acute rejection that could not be controlled with additional immunosuppression and required allograft removal within the first year after transplantation.

Figure 4. Moderate rejection of the small intestinal graft is demonstrated here by villous blunting (small arrows) and abnormal villous architecture (large arrow). The cryptitis seen on higher power has essentially the same appearance as shown in Figure 3 *b*; however, generally more crypts are involved.

Blood Type

Three patients were transplanted with grafts from blood-group-compatible, nonidentical donors. One blood group AB recipient received a small bowel graft from a blood group A donor. She developed only one mild rejection episode 12 days after transplantation and has a functional graft 4.5 yr after transplantation. Two other recipients (blood group B, n = 1; blood group A, n = 1) received small bowel grafts from blood group O donors. Both of these recipients developed multiple rejection episodes and underwent explantation of their bowel grafts for uncontrolled acute rejection 150 and 353 days after transplantation, respectively.

Cross-Match

Three patients were found retrospectively to have positive lymphocytotoxic cross-match results. Two of the three patients with positive cross-matches lost their grafts to acute rejection, although hyperacute rejection was not identified in these recipients. One patient with multiple mild acute rejection episodes after transplantation presented with abdominal pain and pneumatosis intestinalis (10 days after initial hospital discharge). At laparotomy, her entire bowel graft showed full thickness necrosis and was removed. A second patient (patient 8) also had a nonidentical-blood-type donor. She was successfully treated for 10 rejection episodes, but then developed severe rejection 11 months after transplantation and underwent allograft removal. The third patient continues to have good graft function 8 months after transplantation and has had three rejection episodes.

The patient whose graft failed from hyperacute rejection has been previously reported (7). He had negative T- and B-cell cross-matches, but further studies revealed the presence of preformed antibody to endothelial monocyte antigens.

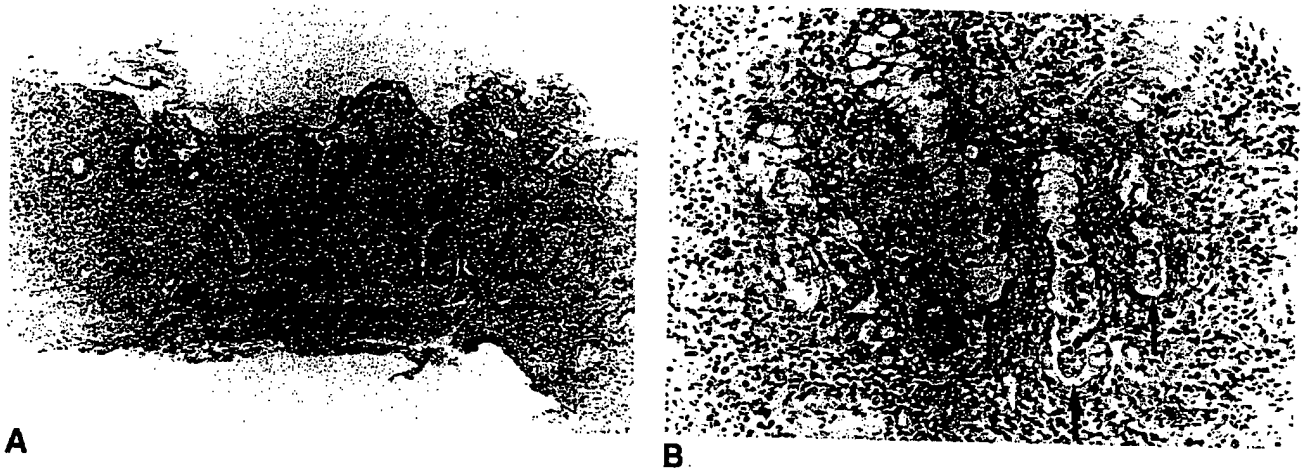

Figure 5. Severe rejection of a small intestinal graft is demonstrated here *(A)* by ulceration of the epithelium and severe blunting of the few remaining intact villi (magnification ×40) and *(B)* by crypt cell destruction and debris (arrows) (magnification ×200).

Liver Dysfunction

Seventeen of the 28 isolated small bowel transplant recipients had evidence of liver dysfunction. Eleven of the 28 isolated small bowel recipients were overtly jaundiced at the time of transplantation, with a median serum total bilirubin of 6.5 mg/dl (range = 3–26). Liver biopsies in these patients demonstrated portal fibrosis with or without bridging in nine (see Tables 3 and 4). In addition, six recipients with normal or near-normal (<2.0 mg/dl) serum bilirubin demonstrated portal or bridging fibrosis on liver biopsy. The decision between isolated intestinal and combined liver/small bowel transplantation in these recipients was based on the absence of established cirrhosis on pretransplant needle liver biopsy and the absence of clinical stigmata of portal hypertension (hepatosplenomegaly, portal hypertensive gastropathy, gastroesophageal varices, dilated superficial abdominal veins), as described earlier. In the 11 overtly jaundiced recipients of isolated small bowel allografts, the serum total bilirubin gradually returned to normal levels 1–4 months after transplantation in all eight with functional grafts. Patient and graft survivals in these 11 recipients were not significantly different from the 17 recipients with normal bilirubin at the time of transplantation (1-yr patient survival 94% *vs* 88%, p = NS and 1-yr graft survival 68% *vs* 73%, p = NS).

Three of the 11 jaundiced patients developed coagulopathy and intraabdominal bleeding in the first week after transplantation. One of these three became hypotensive, which precipitated severe acute liver failure; this patient died 8 days after transplantation. Only one patient without jaundice developed intraabdominal hemorrhage and this patient had been anticoagulated perioperatively because of known protein C deficiency. Two patients (one with portal fibrosis on liver biopsy without jaundice and one jaundiced patient) developed small bowel obstruction from a mural hematoma at the site of small bowel biopsy 2 and 3 wk after transplantation, respectively. Both required resection of the involved graft. No patient without liver dysfunction developed this complication.

Morbidity

The median length of initial intensive care unit stay was 5 days (range 2–92) and the median length of initial hospitalization was 33 days (range 8–217). All 23 small bowel transplant recipients discharged with functional grafts required readmission to the hospital during the first year after transplantation.

All recipients of isolated small bowel transplants suffered complications postoperatively. Surgical complications included wound infection in two recipients, fascial dehiscence in three recipients, and intraabdominal or submucosal hemorrhage in seven recipients (all with liver dysfunction, as noted earlier). Iatrogenic complications occurred in four patients. Complications of endoscopy included disruption of mucosal-cutaneous sutures of the jejunostomy in one, gastric perforation in one, and mural hemorrhage at biopsy sites in two. A small bowel perforation occurred in one patient at the tip of a jejunal feeding tube. Infectious complications were numerous. Eighteen episodes of bacteremia occurred in 15 recipients and could be attributed to central line infection in 8 recipients. In addition, intraabdominal abscess occurred in five recipients.

Seventeen episodes of viral enteritis were identified in 13 recipients: cytomegalovirus (CMV) occurred in three, adenovirus in 11, and rotavirus in three. The diagnosis of CMV infection was made by early antigen test of biopsy tissue and confirmed by tissue culture and histological evidence of viral inclusions. All three occurred in the first 3 months after transplantation (posttransplant day 45, 52, and 90, respectively). All three recipients were seropositive for CMV at the time of transplantation. Six additional recipients were seropositive at the time of transplantation and did not develop CMV enteritis. All three patients with CMV enteritis responded well to decreased immunosuppression and intravenous administration of ganciclovir. Adenoviral infection was diagnosed primarily by culture in 11, supported in some by identification of histological evidence of viral inclusions.

Table 5. Details Regarding the Development, Treatment, and Outcome of PTLD After Small Intestinal Transplantation

Age at Transplantation, Yr	Time After Transplantation, Months	Site	Chemotherapy	Outcome
6	6	Chest mass	Yes	Cure, functional graft
2	5	Maxillary sinus mass	Yes	Cure, rejection, retransplantation
2	3	GI, lymph nodes	Yes	Cure, rejection, awaiting retransplantation

PTLD = posttransplant lymphoproliferative disorder.

Rotavirus infection was confirmed by enzyme assay of ostomy output.

Posttransplant Lymphoproliferative Disorder (PTLD)

Three patients (11%) developed PTLD after small intestinal transplantation, as noted in Table 5. All three patients who developed PTLD were Epstein-Barr virus (EBV) seronegative at the time of transplantation. All developed a localized mass or diffuse adenopathy. Biopsy of the lesions revealed monoclonal B-cell proliferations, which were lambda positive and EBV positive by *in situ* hybridization with an Epstein Barr Early mRNA (EBER) probe. None responded to reduced immunosuppression and antiviral therapy and all were subsequently treated with cyclophosphamide and additional corticosteroids as previously described (8). The three recipients had been treated for two, three, and two rejection episodes, respectively, before diagnosis of PTLD. One of the three patients had received OKT3 for the treatment of acute rejection, as had six of the 20 who did not subsequently develop PTLD (p = NS). One patient has resumed immunosuppression with tacrolimus after completion of chemotherapy and two patients required explantation of their intestinal grafts for severe acute rejection and sepsis while undergoing chemotherapy.

Pretransplant EBV serologies were available in 23 of the 24 recipients with 30-day graft survival. Negative EBV serology was present in 12 patients (52%) at the time of transplantation (including three who developed PTLD). No patient who was seropositive at the time of transplantation developed PTLD. EBV DNA levels were obtained in 14 recipients at various times after transplantation. In seven of the 14, they remained negative at all time points and none of these patients developed PTLD. In the three with PTLD, there was wide variation in the peak levels of EBV DNA (10,000 copies/μg, 1500 copies/μg, and 250 copies/μg, respectively). All three showed decline of the EBV DNA level with initiation of chemotherapy.

Graft-Versus-Host Disease (GVHD)

No isolated small bowel transplant recipient developed GVHD in this series.

HLA Typing

Because there was no attempt to select donors based on HLA typing and there was uniformly poor matching in all recipients (see Tables 3 and 4), no meaningful conclusions can be drawn from the effect that increased matching might have on graft survival.

Retransplantation

Three patients underwent retransplantation after loss of their initial small bowel allograft. The times from explantation to retransplantation were 4 and 10 months, respectively, in two patients whose grafts were lost from uncontrolled acute rejection (patients 8 and 10, see Table 3). These two patients have functional grafts (have been discharged from the hospital on a full enteral diet) and are currently 8 and 22 months, respectively, after retransplantation. The time to retransplantation was 8 days in the patient with hyperacute rejection (patient 6). He again had a negative lymphocytotoxic cross-match, but developed hyperacute rejection of the second allograft requiring explantation within hours of revascularization.

DISCUSSION

Since the introduction of PN, it has been the treatment of choice for patients with intestinal failure. The reasons for this development are clear. First, the early results of intestinal transplantation were dismal (9–12). On the other hand, 1-yr survival rates for patients dependent on PN (excluding patients with cancer and AIDS) are reported to vary from 78% to 97% (2). In light of this, isolated intestinal transplantation has been offered only to those patients with intestinal failure who have life-threatening complications of PN administration.

Since the introduction of tacrolimus, small bowel transplantation has achieved reasonable clinical success. Although the overall experience remains relatively small, the experience of our center and the report of the transplant registry since January 1995 reveal 1-yr patient/graft survival of 93%/71% and 90%/65%, respectively (13). Although patient survival in patients with good graft function in the current era is similar to survival of patients on PN, the major drawback to widespread application remains graft survival. Despite tacrolimus, rejection is an almost uniform occurrence after small bowel transplantation. Rejection was identified in 22 of 23 intestinal transplant recipients with functional grafts in the first year after transplantation in this series. This is much higher than the expected incidence of rejection with cyclosporine- or tacrolimus-based immunosuppression in kidney (33–40%) or liver (50–70%) transplant recipients (14, 15). With these higher rates of acute rejection in small bowel transplant recipients, it is not surprising to find that graft failure from acute rejection is much higher than for other solid organs. In this series, graft failure

from acute rejection occurred in 19% of small bowel transplant recipients. In liver transplant recipients, on the other hand acute rejection was the cause of graft failure in only 1–5% (16, 17). Graft loss from acute rejection even 3 yr after kidney transplantation was only 3–10% (18). The reason for the increased immunogenicity of the bowel in comparison to other solid organs is unclear, but this is an area that would benefit from future investigation.

To decrease the risk of rejection of small intestinal grafts other centers have tried OKT3 induction, Cytoxan, mycophenolate mofetil, or azathioprine in addition to tacrolimus and prednisone, but have failed to show any improvement in graft survival with these regimens (19, 20). In addition, lower patient survival and a higher incidence of PTLD have been noted with the additional immunosuppression (20). The introduction of interleukin-2 (IL-2) inhibitors in the immunosuppressive regimen for kidney transplant recipients has led to a decrease in the incidence of acute rejection, in the range of 10–20% (21, 18). Trials with these new IL-2 inhibitors have not yet been reported in bowel transplant recipients.

Part of the reason for the high rate of graft loss may be attributable to the lack of specificity for the histological changes identified as acute rejection in intestinal allograft biopsies. Furthermore, the distinction between rejection and infectious injuries of the allograft is problematic. For example, pathologists have described changes on intestinal biopsy specimens consistent with acute rejection in patients; these have subsequently grown adenovirus or cryptosporidium from tissue culture. In retrospect, one wonders if the changes were due to the infectious organisms and the rejection mistakenly diagnosed, or whether these processes coexist or even predispose one to the other. Treatment of infectious injuries with increased immunosuppression and failure to treat rejection are both scenarios likely to result in poor outcomes. Perhaps this will become clearer as increased experience is gained.

All patients in this series have discontinued PN and have remained on a completely enteral diet without the need for PN supplementation. There are no grafts in this series with only partial function. Other series have reported a need for supplementary PN in 4–22% of intestinal transplant recipients (22, 23). In one series, the reason for supplemental PN was reported as pseudoobstruction in the native gut. Some have recommended so-called "novel techniques for reconstruction of the proximal alimentary tract" in patients with a history of pseudoobstruction (24). This has been unnecessary in our experience; however, it is unclear at this time whether the improvement in gastric function will be sustained or whether progression of the pseudoobstruction in the remaining native gut will occur. Longer follow-up will clarify this issue.

As in all recipients of solid organ transplants, infection is a common complication in small bowel transplant recipients. In this series, 91% of recipients developed infectious complications in the first year after transplantation. This is comparable to rates published by the group from the University of Pittsburgh (97%) (25). Central line infections are a common preventable source and therefore removal of central venous access catheters as quickly as possible after transplantation should be a high priority. The very high levels of tacrolimus needed to prevent and treat rejection likely contribute to this high rate of infection. Furthermore, a clinical scenario unique to small bowel transplantation is bacteremia and/or fungemia in association with an episode of acute rejection. The bowel injury caused by preservation, rejection, or enteric infection leads to ulceration and denudation of the mucosa when severe. This loss of mucosal integrity appears to allow for translocation of bacteria and other enteric organisms. Several groups have documented the loss of mucosal integrity with nuclear medicine studies revealing increased permeability at the time of rejection and for a short period after treating rejection while the bowel graft is healing (26–28). Physicians caring for intestinal transplant recipients must maintain a high index of suspicion for rejection at the time of identified bacteremia.

Many of the early reports of intestinal transplantation in animals and humans describe a systemic inflammatory syndrome in the early postoperative period with large requirements of intravenous fluids, hemodynamic instability, and pulmonary congestion (29, 9, 31, 32). This syndrome is most likely the result of preservation injury to the bowel, prolonged cold ischemia, or bacterial translocation when the colon is included as part of the graft. With our procurement technique and short cold ischemia times we only saw this syndrome in the patient with diffuse mucosal necrosis from hyperacute rejection.

Intestinal transplant recipients seem to be at higher risk of PTLD (11% in this series) than do recipients of other solid organ transplants (1–5%) (33). This is likely due in part to the high levels of immunosuppression required to prevent or control acute rejection. Furthermore, because many of these transplants are performed in children, a large number of recipients are at risk for the development of primary EBV infections after transplantation. Our center has adopted a prophylaxis strategy using high-dose acyclovir and cytogam after the report by Stratta *et al.* revealed a lower incidence of EBV and other viral infections on this regimen (6). This may have contributed to our lower incidence of PTLD infections compared with other centers (11% *vs* 27%) (20). Our center has also proposed a chemotherapeutic regimen that appears very effective, and may explain the difference in mortality related to PTLD in this series, compared with others (0% *vs* 45%) (8, 20, 34).

Graft-*versus*-host disease was not identified in any patient in this series. Others have reported a 5% incidence of GVHD after small bowel transplantation (20). The lack of GVHD in this series may be attributable in part to our center's protocol treatment of organ donors with antilymphocyte antibody before organ procurement. This treatment clearly causes depletion of donor lymphocytes as demonstrated in this study by the absence of viable lymphocytes

for cross-match and HLA studies in donors where lymph nodes were obtained after removal of the solid organs. Another possible reason for the increased incidence of GVHD in other centers may be the addition of bone marrow infusions in combination with intestinal transplantation (35).

CONCLUSIONS

The 1-yr and long-term patient survival reported here is similar to current survival estimates of all patients with benign disease on PN. Although there is clearly room for improvement, graft survival after isolated intestinal transplantation is acceptable and similar to liver and lung transplants. Small bowel transplantation can sustain the entire nutritional needs of recipients and is associated with complete reversal of liver dysfunction. Caution must be used, however, in the deeply jaundiced patient because of their increased risk of hemorrhage and liver failure. Better immunosuppression is needed to prevent graft loss from acute rejection and to decrease the risk of infection and PTLD. For patients with intestinal failure suffering from life-threatening complications of PN, isolated small intestinal transplantation is an established safe and effective therapy. With better immunosuppression, the time is approaching when small bowel transplantation may be more broadly applied to improve quality of life in PN-dependent patients, without the possibility of life-threatening complications.

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